PLATFORM G: Membrane Structure I

100-Plat
Respiration: An Immiscibility Interfacial Phenomenon
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Pulmonary surfactant (PS) is a surface active lipoproteic material produced by type II cells at the alveoli. This material forms a unique air-liquid interface lining the alveolar surface that reduces surface tension close to 0mN/m, maintaining lung volumes and alveolar homeostasis at the end of expiration. The particular lipid composition of PS suggests that mono- and bilayer-based structures should exhibit lateral phase segregation at physiological temperatures. This work on Native Pulmonary Surfactant Membranes (NPSM), directly isolated from broncho-alveolar lavages of wild-type mice show that a detailed lipid compositional study is crucial to understand the structure and biophysical function of these complex mixtures. Using different microscopy techniques, we have managed to analyze, in detail at the micro- and nano-scale, qualitative and quantitatively the whole native interfacial film responsible for breathing in mice. First, it was performed a study of the lipid phase segregation pattern on free-standing spherical and in planar solid-supported bilayers. Then, the phase behavior was investigated in planar solid-supported and in in-situ air-liquid monolayers. We found close correspondence in shape, size, height, area coverage and lipid phase of the domains between bilayers and monolayers. Contrary to what has been published until now. Particularly with monolayers, the quantification of the order among the different coexisting lipid phases indicates that the segregated rounded domains within a percolated larger area are more fluid. The phase segregation pattern remains until physiological relevant respiratory surface pressures, where we found non-homogeneous nanostructure serving as a platform for a highly corrugated-like collapsed structures, that grow size- and height-wise, arising from the more fluid phases. This last finding opens a new way to explore the pulmonary surfactant interfacial films, closer to materials science, which could help to understand such a basic and important topic; the respiration in mammals.

101-Plat
Fluid Pulmonary Surfactant Membranes of Torpid Animals Possess an Orderly Solid-Like Phase at Low Body Temperatures
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Pulmonary surfactant (PS), a lipo-protein complex, regulates interfacial surface tension of the lung. The fluctuations in body temperatures of heterothermic mammals correlate with fluctuations in surfactant lipid composition as well as function. Previously we speculated that the higher levels of cholesterol during torpor will reduce phase transition temperature (T_m) enabling PS to remain fluid over a broader range of temperatures. However, these compositional changes do not explain how the surfactant can attain low surface tensions without suffering film collapse at the interface. To gain a better understanding of the molecular interactions that take place at the air-water interface, responsible for surfactant to remain surface active at low temperatures, we explored the thermodynamic interactions and phase-transitional behavior of pulmonary surfactant membranes of heterothermic mammals namely fat-tailed dunnarts (*Sminthopsis crassicaudata*) and Gould's wattled bats (*Chalinolobus gouldii*). Thermodynamic studies were conducted with fluorescence spectroscopy by LAURDAN (6-dodecanoyl-2-dimethyl-aminonaphtalene), fluorescence anisotropy by DPH (1, 6-diphenyl-1, 3, 5 hexatriene) and differential scanning calorimetry. We also conducted epifluorescence and atomic force microscopic studies to visualise phase coexistence of surfactant membranes of these animals. Surfactant membranes of torpid animals showed gel-to-fluid transitions at lower T_m and lower enthalpy compared to warm-active animals indicating a more fluid like surfactant. However, at low temperatures, fluorescence spectroscopy and anisotropy studies showed that surfactant from torpid animals possessed a dehydrated solid-like ordered phase similar to that of the warm-active group. This trend was further confirmed by microscopic studies, which revealed structural differences in the morphology and distribution of compression-driven segregated lipid domains in surfactant films. This suggests that in torpid animals, surfactant alters its composition as an adaptation to reduced body temperatures but retains its function by making structural re-arrangements of the surface active film.

102-Plat
Atomic Force Microscopic Examination of Rat Pulmonary Surfactant Films
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The ability of pulmonary surfactant to drive surface tension (ST) to low values near 0 mN/m during compression stabilizes the alveoli at end expiration. The manner in which surfactant phospholipids (PL) (50% unsaturation) attain surface tension near zero is still not clear. Atomic force microscopy (AFM) of rat surfactant extract films at a surface pressure (SP) of 20 mN/m reveals microdomains (mD), apparently composed of liquid-ordered (L_0) and tilted-condensed (TC) phases within the predominant liquid-expanded (LE) phase. Numerous nanodomains (nD) were also present. As SP increased to 40 mN/m, the area composed of mD increased but the nD decreased. This contrasts with previous studies with bovine surfactant where the mD appeared to form nD. At 50 mN/m, the film exhibited the formation of numerous stacked multilayers (PL bilayers) which could be incorporated into the monolayer during film expansion. The present results are consistent with the reversible squeeze-out of unsaturated PL into multilayers during compression, resulting in a monolayer highly enriched in gel phase saturated PL components. Furthermore, as with other surfactants, the presence of L_0 phase appears related to cholesterol. Reducing the cholesterol content by acetone precipitation resulted in the loss of L_0 phase, further implicating this sterol in surfactant phase separation.

103-Plat
The Effect of Surface Tension on the Phase Equilibrium of DPPC/DOPC/Cholesterol Model Lipid Bilayers
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Experimental observations of liquid-ordered (lo) liquid-disordered (ld) phases in living cells have been elusive. However, recent experiments on giant plasma membrane vesicles (GPMVs) that were isolated directly from living cells showed that these GPMVs contain two macroscopic liquid phases, lo and ld, at low temperatures and one liquid phase at high temperatures and exhibit transition temperatures in the range of 15 to 25 °C [Veatch, S. L., et. al., Chem. Bio. Lett., 3, p. 287 (2008)]. The GPMVs are particularly interesting since they retain most of the compositional complexity of living cell membranes. One of the major differences between the lipid bilayer in the GPMVs and the lipid bilayer in the living cell membrane is that the GPMVs are free from the actin cytoskeleton network. It has been shown in renal epithelial cells that tether forces, and therefore the apparent membrane tension, are significantly lower on blebs than on membranes that are supported by the cytoskeleton [Dai, J. and Sheetz, M. P., Biophysical Journal, 77, p. 3363 (1999)].
Using a theoretical model of a bilayer membrane containing cholesterol, dipalmitoyl-phophatidylcholine (DPPC), and dioleoylphosphatidylcholine (DOPC) that qualitatively reproduces phase diagrams of tensionless GUVs of the same three components [R. Elliott, I. Szleifer, and M. Schick., Phys. Rev. Lett., 96, p.098101 (2006)], we show that increasing the tension on the lipid bilayer changes the phase diagram dramatically. Increasing the tension on the bilayer changes the location of the lo-ld transition until at a high enough tension the liquid-liquid phase equilibrium disappears. This leads us to speculate that the lipid bilayers connected to the actin cytoskeleton in living cells are not separated into two liquid phases due to the enhanced tension provided by the cytoskeleton.

104-Plat
Chemical Imaging of Lipid Organization in the Plasma Membranes of Intact Cells with High Lateral Resolution
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Compartmentalization of the plasma membrane into domains of differing composition is required for proper cell function, but the extent and origins of this organization is poorly understood. The non-random distributions of